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Prelimbic medial prefrontal cortex disruption during adolescence increases susceptibility to helpless behavior in adult rats

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Abstract

Major depressive disorder (MDD) is a disabling mental disorder worldwide. Several animal models have been used to study the neurobiology of this disorder, including the learned helplessness (LH) paradigm, in which susceptible animals show helpless behavior indicated by fails to escape a controllable footshock. This behavior has been associated with a downregulation of ventral tegmental area (VTA) dopamine (DA) system activity. The prelimbic portion of the prefrontal cortex (pIPFC) plays an important role in the modulation of helpless behavior, but so far there is no evidence indicating that its developmental disruption alters susceptibility to helpless behavior. We investigated the impact of pIPFC lesion performed at adolescence (postnatal day 31–33) or adulthood (postnatal day 70–72) on anxiety responses (elevated plus-maze), susceptibility to helpless behavior, and the VTA DA system activity in adult Sprague-Dawley rats. Whereas adult pIPFC lesions induced neither anxiety responses nor increased susceptibility to helpless behavior (pIPFC lesion: 33.3% of helplessness; controls: 30.8% of helplessness rats), adolescent pIPFC lesions induced anxiety responses and increased the proportion of rats showing helpless at adulthood (pIPFC lesion: 92.3% helplessness; controls: 42.1% helplessness rats). Moreover, only helpless rats in the groups showed a decreased VTA DA system population activity that was confined to the medial portion of the VTA. These findings suggest that the impairment of pIPFC activity during adolescence occurs during a critical window for the development of helpless

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Author Contributions

Daniela L Uliana: conceptualization, methodology, formal analysis, investigation, writing - original draft. Felipe V Gomes: conceptualization, methodology, investigation and writing - review & editing. Anthony A Grace: conceptualization, resources, writing - review & editing, supervision and funding acquisition.

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Statement of Interest

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behavior in adult rats, indicating that predisposition or early life adverse events that impair pIPFC activity may enhance susceptibility to depression in adulthood.

Keywords

depression; dopamine; prefrontal cortex; adolescence

1. Introduction

Major depressive disorder (MDD) is a disabling disorder with high prevalence worldwide (Kessler and Bromet, 2013). MDD is complex and involves a multitude of symptoms, such as depressed mood, loss of interest or pleasure (i.e., anhedonia), fatigue, and sleep problems for 2 weeks or more (American Psychiatric Association, 2013). An important external factor that can predict the development of depression is stress (Richter-Levin and Xu, 2018). Several animal models based on stressful situations are used to study depression, such as chronic mild stress (CMS), social defeat, and learned helplessness (LH) (Czéh et al., 2016; Muir et al., 2018). The LH paradigm, in particular, is based on the inability of the individual to escape from an aversive controllable situation due to a previous unpredictable/uncontrollable stress (Maier and Seligman, 2016; Seligman and Maier, 1967). It is proposed that LH induces behavioral changes that have some similarity with those observed in depressive patients, such as anhedonia (Sanchis-Segura et al., 2005).

Anhedonia and helpless behavior have been associated with a decreased activity of the dopamine (DA) reward system (Nestler and Carlezon, 2006; Wise, 2008). Rats undergoing LH, CMS and cold stress models for depression all show a hypodopaminergic state indicated by a decrease in the activity of ventral tegmental area (VTA) DA neurons (Valenti et al., 2012; Chang and Grace, 2014; Moreines et al., 2017; Rincón-Cortés and Grace, 2017; Douma and de Kloet, 2019; Kaufling, 2019). In addition, optogenetic inhibition of the activity of VTA DA neurons has been described to induce a depression-like phenotype (Tye et al., 2013). Therefore, the downregulation of the DA system seems to be critically related to a depressive-like phenotype, including helpless behavior (Belujon and Grace, 2017, 2014).

Of the many brain regions involved in the regulation of stress responses, the medial prefrontal cortex (mPFC) plays a prominent role (McEwen and Morrison, 2013). There are two subdivisions of the mPFC that have been linked with stress and affect regulation. The infralimbic PFC (ilPFC) exhibits an excitatory projection to the amygdala (Likhtik et al., 2006) and activation of this pathway leads to increased anxiety (Radley et al., 2006) and with repeated stress, depression-like behaviors (Chang and Grace, 2014). In contrast, the prelimbic PFC (plPFC) has projections to GABAergic neurons in the amygdala (Rosenkranz and Grace, 2002) and has been shown to attenuate amygdala responsivity to stressors (Figueiredo et al., 2003; Jones et al., 2011; Radley et al., 2006; Rosenkranz and Grace, 2002). Thus, a plPFC dysfunction could increase amygdala responsivity to stress, which in turn could facilitate the impact of stress. We found previously that adolescent plPFC lesions increased vulnerability to the development of schizophrenia-like changes induced by stress

during adolescence in rats (Gomes and Grace, 2017). Concerning depression, studies investigating LH neurobiology demonstrate an important role of pLPFC activity in detecting stress controllability via inputs to the dorsal raphe nucleus (DRN) (Amat et al., 2008, 2005; Maier and Seligman, 2016), indicating that the pLPFC provides a top-down control over helpless behavior.

Adolescence represents a critical period in the maturation of the PFC (Caballero and Tseng, 2016) and adversities experienced during this period of neurodevelopment have a profound impact on the emergence of several psychiatric disorders, including depression (Andersen and Teicher, 2008; Brenhouse and Andersen, 2011; Spear, 2013; Keshavan et al., 2014; Marín, 2016). However, there is no evidence examining whether developmental disruption of the pLPFC affects susceptibility to helpless behavior. Thus, we investigated the impact of pLPFC lesion, performed at adolescence or adulthood, on the susceptibility to helpless behavior and its corresponding effects on VTA DA system activity in adult Sprague–Dawley rats.

2. Experimental procedures

2.1. Animals

Sixteen pregnant Sprague–Dawley rats (Envigo, Indianapolis, USA) were obtained on gestational day 14 and each pregnant rat was housed individually in ventilated plastic breeding tubs. Male offspring (81 rats) were weaned on postnatal day (PD) 21 and housed in groups of two or three with littermates. Male rats were used in this study to match prior work; future work extending to females will require study into the precise timing of the insult given differences in puberty onset and susceptibility to LH. Forty-two rats at PD 31–33 were subjected to the pLPFC lesion surgery (24 sham and 18 lesioned animals) and used for behavior/electrophysiology experiments at adulthood (>PD65). Thirty-nine adult rats at PD 70–72 were subjected to adult pLPFC lesion surgery (19 sham and 20 lesioned rats) and tested for behavior/electrophysiology after at least 10 days of recovery. All rats were housed under standard housing conditions: 12h/light-dark cycle (regular light cycle: lights on at 7 a.m.; reverse light cycle: lights on at 7 p.m.) and temperature-controlled room ($22\pm 1^\circ\text{C}$), with free access to food and water. Behavioral experiments were performed during the lights-off cycle. The procedures were carried out following the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

2.2. Experimental Design

We tested whether a lesion of the pLPFC during either adolescence or adulthood would increase the susceptibility of adult Sprague–Dawley rats to LH. The pLPFC lesion was induced by infusing ibotenic acid bilaterally into the pLPFC in rats at PD31–33 (adolescence) or PD70–72 (adulthood). As adults, rats were tested in the elevated plus-maze (EPM) and then subjected to LH two days later. Four days after LH, activity patterns of VTA DA neurons were assessed. A separate group of animals that received saline (sham) or ibotenic acid injection during adolescence (PD31) and adulthood (PD70) were tested in the EPM and the VTA DA recordings at adulthood, without being exposed to LH. The injections were

randomized by experimenters and blinded for the behavioral and electrophysiological experiments.

2.3. pIPFC lesion

The rats were anesthetized with isoflurane and fixed in a stereotaxic frame. Ibotenic acid (5µg/0.5µl; Sigma) or saline (0.9% NaCl) was infused bilaterally (0.5µl) into the pIPFC using a 10µl Hamilton syringe and infusion pump (11 plus Harvard apparatus; Rate: 200µl/min). The pIPFC coordinates used in adolescent rats were: +2.9 mm antero-posterior, ±0.5 mm mediolateral from bregma, – 3.1 mm ventral from the skull surface, these coordinates were based on a previous study from our group (Gomes and Grace, 2017); and for adults: + 3.2 mm antero-posterior, ± 0.7 mm mediolateral from bregma, – 4.1 mm ventral from skull surface, according to Paxinos and Watson (2007) (Paxinos and Watson, 2007). A subcutaneous injection of the nonsteroidal anti-inflammatory carprofen (Rimadyl, Zoetis Inc) was administered for post-operative analgesia at the end of the surgery. Medicated food (MediGel@CPF) was also provided in the rat homecage. The animals were kept together with their respective littermates during recovery. Histological verification of the lesion was evaluated at adulthood (Figure 1A–B), after the electrophysiology recordings of DA neurons in the VTA.

2.4. Elevated plus-maze (EPM)

The EPM consisted of two opposite open (50 cm × 10 cm) and enclosed (40 cm high opaque wall) arms crossed at a right angle and located 50 cm above the floor. In the test, rats were placed in the center of the maze facing an enclosed arm. The test was recorded over 5 minutes and the percentage of time and entrances into the open arms were measured as an index of anxiety. The number of total entries was evaluated as an index of locomotor activity.

2.5. Learned Helplessness (LH)

The LH paradigm has been widely used as a model of stress inducing depression-like behaviors and was based on a previous study from our group (Belujon and Grace, 2014). The apparatus consisted of a two-chamber shuttle box with grid floor and stainless-steel rods plugged into a shock generator (Med Associates, USA). On day 1, the rats were subjected to inescapable stress session, consisting of 120 inescapable and uncontrollable footshocks (1 mA; 7–22 s interval duration; 35–85 s interval between the footshock) in one side of the chamber. In the next day, the animals were tested for helpless behavior in the active avoidance session. The rats were first acclimated to the shuttle box for 5 minutes (both sides), following by 25 trials of unexpected and escapable footshock. The trial started with a 5-second tone and a light followed by a 0.8 mA footshock. The termination of shock required two crossings in the shuttlebox (FR2) or a maximum of 15 seconds in case of no crossing response. The number of escape failures and the mean escape latency were evaluated during 25 FR2 trials. More than 10 escape failures and 8 seconds of the mean latency to escape were used as a criterion for helpless behavior, according to a previous study by our group (Belujon and Grace, 2014).

2.6. *In vivo* extracellular VTA DA neuron recordings

Rats were anesthetized with chloral hydrate (400mg/kg, i.p., Sigma), following by fixation in the stereotaxic frame. A thermostatically-controlled heating pad was used to maintain body temperature at 37 °C. Electrodes pulled from Omegadot 2.0 mm glass tubing were used for *in vivo* extracellular recordings of DA neurons in the VTA. The electrode tip was broken back under microscopic control to an impedance of 6–16 MΩ and filled with 2M NaCl containing 2% Sky Blue dye (Sigma). The electrodes were lowered into VTA (– 5.3 mm posterior from bregma, 0.6 mm lateral to the midline and 6.5–9.0 mm ventral from brain surface). Six to nine vertical tracks were sampled within the VTA in a fixed pattern (Figure 2D, Belujon and Grace, 2014). The parameters evaluated were: population activity (number of spontaneously active DA neurons per track), firing rate and percentage of spikes occurring in bursts. These parameters were also evaluated in relation to medial, central and lateral portions of VTA. The identification of DA neurons was based on well-established electrophysiology characteristics (Figure 2, Grace and Bunney, 1983; Ungless and Grace, 2012). In the VTA recordings, some animal data were excluded due to electrode misplacement. Five rats (3 from the saline group and 2 from the ibotenic acid group) were excluded in the adolescent lesion experiment due to incorrect electrode placements.

2.7. Histology

The electrode location was marked with an electrophoretic ejection of Chicago Sky Blue dye (–20μA constant negative current, 20 min) at the end of VTA recordings (Figure 2E). Euthanasia was induced by the administration of a lethal dose of chloral hydrate (additional 400mg/kg).

The rats were decapitated and the brains removed. The brains were fixed in 8% paraformaldehyde for approximately 48h followed by 25% sucrose solution for cryoprotection. After saturation, the brains were frozen and sliced coronally (PFC, 40 μm; VTA, 60 μm) using a cryostat (Leica Frigocut 2800). PFC and VTA slices were mounted on gelatin-coated slides, followed by staining with a combination of neutral red and cresyl violet.

2.8. Statistical Analyses

Behavior and electrophysiology data were expressed as mean ± SEM and analyzed using Student's t-test or 2-way ANOVA with lesion (ibotenic acid or saline) and condition [(1) not exposed to the LH and (2) exposed to the LH - helplessness (3) exposed to the LH - nonhelplessness] as main factors followed by the Tukey's post hoc test. The percentage of rats showing helpless behavior was analyzed using the Chi-square test. $p < 0.05$ was defined as significant.

3. Results

3.1 Adolescent pIPFC lesion promotes anxiety and increases susceptibility to helpless behavior in adulthood.

The extents of the pIPFC lesions performed during adolescence or adulthood are illustrated in Figure 1E–F. The pIPFC lesion was equivalent in both nonhelpless and helpless rats in the

adolescent and adult experiments. Similar to our previous findings with juvenile (PD24) pIPFC lesions (Gomes and Grace, 2017), we found that the pIPFC lesion at PND 31–33 (n=18) induced anxiety responses at adulthood, as indicated by a decrease in the percentage of time ($t_{40}=4.01$, $p<0.05$, t-test, Figure 3A) and entries into the open arms in the EPM compared to controls (n=24; $t_{40}=3.27$ $p<0.05$, t-test, Figure 3B). These changes were not driven by altered locomotor activity since no significant difference was observed for the number of entries into all arms of the EPM ($p>0.05$, t-test, Figure 3C). Concerning LH, adolescent pIPFC lesions increased susceptibility to helpless behavior at adulthood ($z=7.52$, $p<0.05$, Chi-square test, Figure 3D). Whereas the percentage of adult rats with pIPFC lesions during adolescence showing helpless behavior was 92.3% (12 of 13 total rats), only 42.1% of sham rats (8 of 19 total rats) developed helpless behavior. Adolescent pIPFC lesions (n=13) increased the latency to escape ($t_{30}=2.92$, $p<0.05$, t-test, Figure 3F) and the number of escape failures ($t_{30}=2.82$, $p<0.05$, test-t, Figure 3E) compared to the saline-injected group (n=19). In addition, 2-way ANOVA indicated an effect of the condition for the latency to escape ($F_{1,28}=40.76$, $p<0.05$) and number of escape failures ($F_{1,28}=78.63$, $p<0.05$), but with no effect of the lesion when the animals were separated based on the behavior ($p>0.05$) or an interaction between condition and lesion ($p>0.05$). And, as expected, post-hoc analysis showed an increase in the latency to escape ($p<0.05$; Tukey's test) and the number of escape failures ($p<0.05$; Tukey's test) in helplessness rats compared to nonhelplessness rats in both groups (Figure 3E and 3F).

Regarding VTA DA system activity, 2-way ANOVA showed an effect of the condition for number of spontaneously active DA neurons in the VTA ($F_{1,33}=8.61$, $p<0.05$), but with no effect of the lesion ($p>0.05$) or an interaction between condition and lesion ($p>0.05$). In addition, post-hoc analysis indicated a reduction in VTA DA neuron population activity in adult rats with adolescent pIPFC lesion exposed to LH (n=11 rats; 44 cells; 0.65 ± 0.6 cells/track; $p<0.05$, Tukey's test, Figure 4A) compared to sham animals exposed to LH (n=16 rats; 97 cells; 0.99 ± 0.1 cells/track). In contrast, no difference was observed between sham (n=5; 39 cells; 1.11 ± 0.04 cells/track) and adolescent pIPFC lesioned animals that were not exposed to LH (n=5; 46 cells; 1.22 ± 0.11 cells/track; Figure 4A).

In addition, the firing rate and percentage of spikes in bursts of identified VTA DA neurons were not different among the groups (all factors, $p>0.05$, 2-way ANOVA, Figure 4B–C). When we analyzed the data on the VTA DA system activity to evaluate changes among animals not exposed to LH, nonhelplessness, and helplessness animals, as previously reported (Belujon and Grace, 2014), we found that helpless rats, in both groups (with lesion or not), showed a reduction of VTA DA neuron population activity (condition: $F_{2,31}=8.20$, $p<0.05$; lesion: $p>0.05$; interaction: $p>0.05$; 2-way ANOVA). Animals with adolescent pIPFC lesion showing helpless behavior had a decreased number of spontaneously active VTA DA neurons (n=10; 39 cells; 0.58 ± 0.06 cells/track) compared to naive rats (not subjected to LH) and nonhelplessness rats ($p<0.05$, Tukey's test, Figure 4D). There was also a trend for a reduction in the number of spontaneously active VTA DA neurons in helplessness rats that received saline intra-pIPFC during adolescence (n=7; 33 cells; 0.78 ± 0.15 cells/track; $p=0.05$, Tukey's test) compared to nonhelplessness rats (n=9; 64 cells; 1.22 ± 0.13 cells/track). No significant difference was observed in the average firing rate and percentage of spikes in bursts between helpless and non-helpless animals of sham and

lesioned groups ($p > 0.05$ for condition, lesion, and interaction between condition and lesion, 2-way ANOVA, Figure 4E–F). The analysis of the distribution of active DA cells/track among the medial, central and lateral portions of the VTA demonstrated that the decreased VTA DA neuron population activity observed in either sham or lesioned helpless rats was confined to the medial portion (condition: $F_{2,31} = 4.59$, $p < 0.05$; lesion, $p > 0.05$; interaction, $p > 0.05$; 2-way ANOVA, $p < 0.05$ helplessness rats vs. nonhelplessness rats and animals that were not subjected to LH for sham and lesioned rats, Tukey's test, Figure 4G) No effect was observed in the average firing rate and burst activity of the DA neurons recorded in the medial portion of the VTA (all factors, $p > 0.05$, 2-way ANOVA, Figure 4H–I). Also, no change in the number of active DA neurons, firing rate, and burst activity was found in the central and lateral portions of the VTA (all factors, $p > 0.05$, 2-way ANOVA, Figure 4G–I).

3.2 The adult pIPFC lesion did not change susceptibility to LH.

The adult pIPFC lesion ($n = 20$) did not induce anxiety responses as indicated by no change in the percentage of time ($p > 0.05$, t-test, Figure 5A) and entries into the open arms in the EPM compared to controls ($n = 19$; $p > 0.05$, t-test, Figure 5B). No change was observed in the number of total entries into all arms of the EPM as well ($p > 0.05$, t-test, Figure 5C), indicating that the adult pIPFC lesion did not change locomotor activity. In addition, the adult pIPFC lesion did not change the percentage of rats susceptible to helpless behavior ($p > 0.05$, Chi-square test, Figure 5D). The percentage of rats with adult pIPFC lesion showing helpless behavior was 33.3% (5 of 15 rats), while the percentage of sham rats showing helpless behavior was 30.8% (4 of 13 rats). These findings indicate that the adult pIPFC lesion did not increase susceptibility to helpless behavior. Moreover, adult pIPFC lesion ($n = 15$) did not alter the latency to escape ($p > 0.05$, test-t, Figure 5F) and the number of escape failures ($p > 0.05$, t-test, Figure 5E) compared to saline-injected rats ($n = 13$). 2-way ANOVA indicated an effect of the condition for the latency to escape ($F_{1,24} = 148.4$, $p < 0.05$) and number of escape failures ($F_{1,24} = 149.5$, $p < 0.05$), but with no effect of the lesion ($p > 0.05$) or an interaction between condition and lesion ($p > 0.05$). And, as expected, post-hoc analysis showed an increase in the latency to escape ($p < 0.05$; Tukey's test) and the number of escape failures ($p < 0.05$; Tukey's test) in helplessness rats compared to nonhelplessness rats (data not shown).

For the recordings of VTA DA neurons, a 2-way ANOVA showed no effect of the condition ($p > 0.05$), lesion ($p > 0.05$) or an interaction between condition and lesion ($p > 0.05$), indicating that the adult pIPFC lesion did not affect the number of spontaneously active DA neurons in the VTA ($n = 15$ rats; 90 cells; 0.81 ± 0.05 cells/track; Figure 6A) compared to sham animals exposed to LH ($n = 13$ rats; 87 cells; 0.89 ± 0.09 cells/track) or to sham ($n = 6$; 48 cells; 0.99 ± 0.08 cells/track) and adult pIPFC lesioned animals that were not exposed to LH ($n = 5$; 46 cells; 1.09 ± 0.14 cells/track; Figure 6A–C). The firing rate and percentage of spikes in bursts of identified VTA DA neurons were not different among all groups (all factors, $p > 0.05$, 2-way ANOVA, Figure 6B–C). Helpless rats, in both lesioned and non-lesioned groups, showed a significant reduction of VTA DA neuron population activity (condition: $F_{2,31} = 8.20$, $p < 0.05$; lesion: $p > 0.05$; interaction: $p > 0.05$; 2-way ANOVA; Figure 6D). pIPFC lesioned rats showing helpless behavior ($n = 5$; 28 cells; 0.44 ± 0.09 cells/track) had a decreased number of spontaneously active VTA DA neurons compared to naive rats (not

subjected to LH; $p < 0.05$, Tukey's test) and nonhelpless lesioned rats ($n=10$, 62 cells, 0.91 ± 0.04 cells/track). Also, there was a reduction in the number of spontaneously active VTA DA neurons in helpless rats that received saline intra-pIPFC ($n=4$; 15 cells; 0.54 ± 0.08 cells/track; $p < 0.05$, Tukey's test) compared to nonhelpless sham rats ($n=9$; 72 cells; 1.04 ± 0.09 cells/track). No significant difference was observed in the firing rate and percentage of spikes in bursts between helpless and non-helpless animals of sham and lesioned groups (all factors, $p > 0.05$, 2-way ANOVA, Figure 6E–F). The analysis of the distribution of active DA cells/track in relation to region of the VTA indicated a significant reduction of DA neurons firing in the medial portion of the VTA of either sham or lesioned helpless rats (condition: $F_{2,33}=7.24$; lesion, $p < 0.05$; interaction, $p < 0.05$, 2-way ANOVA, $p < 0.05$ helplessness rats vs. nonhelpless rats and animals that were not subjected to LH for sham and lesioned rats Tukey's test, Figure 6G). No effect was observed in the firing rate and burst activity of the DA neurons recorded in the medial portion of the VTA (all factors, $p > 0.05$, 2-way ANOVA, Figure 6H–I). Also, no change in the number of active DA neurons, firing rate, and burst activity was found in the central and lateral portions of the VTA (all factors, $p > 0.05$, 2-way ANOVA, Figure 6G–I). Thus, in summary, all helpless animals independent of treatment condition exhibited reduced VTA DA neuron population activity.

4. Discussion

The present study demonstrated that disruption of the pIPFC in adolescent rats increases, at adulthood, anxiety and the susceptibility to helpless behavior in the LH paradigm, and the helpless behavior was accompanied by a decrease in the number of spontaneously active DA neurons in the medial VTA. In contrast, the adult pIPFC lesion did not impact anxiety or susceptibility to helpless behavior. Together, these data suggest that adolescence represents a sensitive period for the development of susceptibility to depressive-like changes and PFC maturation since a PFC disruption during this period lead to an increased late vulnerability to helpless behaviors.

PFC dysfunction has been described in depression in humans (Drevets et al., 2008; Rogers et al., 2004). Similarly, the involvement of the PFC in depression-like states has also been demonstrated in animal models. In particular, the pIPFC plays an important role in the modulation of behavioral changes induced by LH (Amat et al., 2008, 2005; Maier and Seligman, 2016), which has been associated with the involvement of the pIPFC activity in the detection of stress controllability in the first-day session of this model. In the LH model, the stress session on day 1 can be controllable or uncontrollable, which affects the animal response in the escape session (day2). The animal is more susceptible to helpless behavior when they do not detect control during the stress session. Thus, the detection of stress controllability determines the animal's action, suggesting that a previous intense and uncontrollable stress generally leads to incapacity and inability to act in future aversive events (Maier and Seligman, 2016). It is proposed that in LH, pIPFC activity during the first session of stress detects controllability via direct projections to the DRN that ultimately inhibits this nucleus (Amat et al., 2005). pIPFC inactivation during controllable stress (escapable shocks) leads to helpless behavior (Amat et al., 2005) and its activation during uncontrolled stress (inescapable shocks) does not promote this behavior (Amat et al., 2008), demonstrating the importance of the pIPFC in top-down control over helpless outcome. We

performed the experiment using only the uncontrollable stress paradigm and not controllable stress in day 1, because the pPFC inactivation in adult rats during controllable stress has already been reported to affect the escape session on the following day (Amat et al., 2005), and this could be a confounding factor for interpretation of susceptibility to helpless behavior in pPFC lesion rats. Overall, a pPFC dysfunction could impair the detection of stress controllability during the escape session and increase helpless behavior. Therefore, we hypothesized that a developmental pPFC dysfunction impairs the vulnerability to helpless behavior later in life. This was indeed demonstrated by our findings showing increased susceptibility to helpless behavior in animals with adolescent pPFC lesions. Thus, the increased susceptibility to helpless behavior in animals with adolescent lesion of the pPFC indicates that neurodevelopmental changes in the PFC may increase the susceptibility of an individual to develop depression later in life after exposure to stressful events. Interestingly, the adult pPFC lesion did not increase the susceptibility to helpless behavior or induce an anxiety-like response; these findings are similar to those showing no change in the escape session of the LH when the pPFC is inactivated prior to the exposure to uncontrollable stress (Amat et al., 2005).

The animals subjected to surgery during either adolescence or adulthood did not demonstrate a substantial difference in susceptibility to helpless behavior when compared to the saline groups (42% in adolescence and 30.77% in adulthood), suggesting that the timing of the surgery is not the factor leading to an increased expression of helpless behavior. It is also worth noting that while adolescent pPFC lesioned animals started testing 33 days post-lesion, adult pPFC lesioned animals started testing 10 days post-lesion. Although we did not observe any change in anxiety and helpless behavior 10 days post-lesion in adult-lesioned rats and it is unlikely that these animals would present behavioral changes 30 days post-lesion due to the functional reorganization that is described to take place after the excitotoxic lesion induced by ibotenic acid. In fact, it has been shown that this functional reorganization may promote adaptive mechanisms to regulate stress response and behavior (Vaidya et al., 2019). In the case of the adolescent pPFC lesion, despite some functional reorganization that can take place, we believe that the lesion at this period may impact pPFC maturation leading to a developmental dysfunction of this brain region which in turn drives the increased susceptibility to helpless behavior later in life.

The PFC represents one of the last brain areas to mature in rats, and it happens mainly in late adolescence (Caballero et al., 2016; Davey et al., 2008; Gogtay et al., 2004). In addition, MDD can develop during adolescence (Kessler et al., 2001) and the early onset is related to a greater illness burden throughout life (Zisook et al., 2007). The adolescence period also represents a time in which the connectivity of the PFC with other cortical and subcortical areas emerge, achieving an optimal excitatory-inhibitory balance (Caballero et al., 2016; Caballero and Tseng, 2016; Zimmermann et al., 2019). The pPFC lesion during this period would impair maturation and also remove its connectivity with other structures, diminishing its role in stress controllability and increasing the vulnerability to adverse events and their consequences during development. In this context, it was found that alterations in cortical neurodevelopment are associated with the duration of depression and the number of depressive episodes in humans (Schmitgen et al., 2019). Other evidence points to PFC dysfunction during adolescence in the emergence of depression. The onset of early

depression and PFC maturation share the same period during adolescence (Davey et al., 2008). We found that only 1 of 13 rats with adolescent PFC lesion did not become helpless after the exposure to LH at adulthood. Although this of course precludes appropriate statistical analysis, this reinforces the idea that lesion of the PFC during adolescence leads to a greater vulnerability to stress-induced depression phenotype at adulthood. In addition, similar to what we observed previously (Gomes and Grace, 2017), our results demonstrated that adolescent pIPFC lesion also induced anxiety-like responses in the EPM at adulthood. Anxiety is a common comorbid condition associated with depression (Gaspersz et al., 2018).

The pIPFC is described as a key brain area in the modulation of the stress response, mostly by attenuating the deleterious effects of stress (Arnsten, 2015; Datta and Arnsten, 2019; McEwen and Morrison, 2013). This has been associated with the inhibitory influence that the pIPFC has on amygdala activity, particularly in the basolateral amygdala (BLA) in rats (Hariri et al., 2003; Rosenkranz and Grace, 2002, 2001). In humans, the PFC attenuates emotional responses mediated by the amygdala (Hariri et al., 2003), and PFC dysfunction is related to hyperactivity of the amygdala in depressed patients (Murray et al., 2011). In addition, individuals with cognitive vulnerability to depression exhibit PFC hypoactivation and amygdala hyperactivation in response to emotional stimuli (Zhong et al., 2011). These data suggest that the PFC-BLA pathway is involved in neurobiological changes in depressed patients and that a PFC dysfunction may alter amygdala responsivity to stress which in turn can lead to maladaptive behaviors. Moreover, adolescence is a critical period for the development of the amygdala and PFC and also their connectivity in humans, which increases with age (Gee et al., 2013; Perlman and Pelphrey, 2011; Tottenham and Gabard-Durnam, 2017), and dysfunction in these areas can have a great impact on the development of affective disorders later in life. Young adults with early-onset depression demonstrate amygdala hyperactivity to threat-related faces and this activity is associated with symptom severity (Mingtian et al., 2012). Increased activity of the amygdala also has been described in adolescents with depressive symptoms (Beesdo et al., 2009; Yang et al., 2010; Forbes et al., 2011; Hall et al., 2014), demonstrating that amygdala hyperactivity during adolescence might represent a vulnerability state to the development of depression. Therefore, while certainly not conclusive, these data strongly implicate the amygdala-pIPFC in this effect.

Regarding mPFC-BLA connectivity maturation in rodents, a large increase in fiber proliferation between PD10–30 has been reported (Arruda-Carvalho et al., 2017), reaching a maximal pruning and fiber density at PD25 that remains stable until PD45 (Cressman et al., 2010). After PD45 the anatomical connectivity decreases until adulthood (Arruda-Carvalho et al., 2017; Cressman et al., 2010). Functionally, the mPFC-BLA connectivity seems to achieve an adult-like state around PD40 (Arruda-Carvalho et al., 2017; Selleck et al., 2018). Therefore, these data suggest that the maturational window of PFC-BLA connectivity starts around PD25, supporting that the time-point used in our study to perform the pIPFC lesion (PD31) may represent an important neurodevelopmental period for pIPFC-BLA connectivity. Thus, the increased susceptibility to helpless behavior in animals with adolescent pIPFC lesion may be a consequence of a pIPFC inability to control amygdala responsivity to stress during this period. Consequently, changes in intrinsic PFC activity or connectivity can be essential mechanisms to promote the increased susceptibility to a depression-like state.

Stress during adolescence has a negative impact on PFC, hippocampus, and BLA anatomy, and could influence the emergence of psychiatric disorders by affecting the neurodevelopmental state of the PFC (Andersen and Teicher, 2008; Keshavan et al., 2014; Romeo, 2017). The changes induced in the PFC by social stress in adolescence are reported to be long-lasting in rats (Eiland et al., 2012; Leussis et al., 2008). However, the exact consequence of other stress types during adolescence on adult pathology is still not clear. We previously found that pIPFC lesions in juvenile rats (PND 24) together with mild stress during adolescence (PD31–40) led to schizophrenia-like changes, including an increased population activity of DA neurons projecting to the associative striatum (Gomes and Grace, 2017). In these experiments, we show that adolescent pIPFC disruption increases susceptibility to a depression-like state after exposure to stressful events in adulthood but without adolescent stress exposure. Altogether, these findings indicate that pIPFC dysfunction during the juvenile/adolescence period can increase the impact of stress on susceptibility for the development of schizophrenia or depression, with the timing of the insult determining the pathology.

Regarding the changes in the VTA DA system, it was observed that acute inactivation of the pIPFC led to decreased VTA DA population activity (Patton et al., 2013), but the juvenile pIPFC lesion did not change the VTA DA system in adult animals (Gomes and Grace, 2017), similar to our pIPFC lesion data in adolescent rats. It is possible that the lesion of the pIPFC could promote an adaptation in the pathways involved in the control of dopaminergic activity in the VTA during basal conditions. However, adolescent pIPFC lesioned or sham animals showing helpless behavior presented a decreased VTA DA neuron population activity. Therefore, the pIPFC lesion during adolescence demonstrated an extensive impact on the DA system; an effect that is probably due to the increased susceptibility to helpless behavior that was observed in this group. This is consistent with previous results from our group showing that the reduction in dopaminergic activity in the VTA consistently occurs with helpless behavior in the LH model (Belujon and Grace, 2014), with rats subjected to CMS (Chang and Grace, 2014; Moreines et al., 2017; Rincón-Cortés and Grace, 2017; Neves and Grace, 2019) and also with a combination of repeated footshock/restraint stress during adulthood (Gomes et al., 2019). Thus, we have shown that changes related to depression in rats are linked to the hypodopaminergic state in the VTA, similar to the downregulation of the DA system that is described in depression patients (Lambert et al., 2000; Nestler and Carlezon, 2006; Reddy et al., 1992). Moreover, we also found that the downregulation of the DA system is restricted to the medial portion of VTA in helplessness rats in both treatment groups and independent of the timing of the surgery. This is consistent with previous data from our group demonstrating that in animal models to study depression there is a reduction in active DA neurons in more medial regions of the VTA (Belujon and Grace, 2014; Moreines et al., 2017). The medial VTA is known to project to the nucleus accumbens shell, an area involved with reward-related events (Grace, 2016; Ikemoto, 2007); thus the reduction of the spontaneous active DA cells in the medial portion of the VTA in helpless rat may play an essential role in the reward-related behavioral consequences in the LH model. Supporting this idea, the reduction in the DA reward system is also consistent with the presence of anhedonia previously described in animals subjected to CMS (Tye et al., 2013) and LH (Sanchis-Segura et al., 2005), and support the translational neurobiological changes

in animal models for the study of depression (Wise, 2008). We did not test the reward sensitivity in our study after LH. Nonetheless, the downregulation of dopaminergic activity in the medial portion of VTA can indirectly support the idea of a decreased reward response, as demonstrated by previous studies mentioned above.

In conclusion, our results support the role of pPFC dysfunction in susceptibility to the development of anxiety- and depressive-like behaviors during adulthood. These data reinforce the idea that adolescence represents a sensitive period of vulnerability for the development of psychiatric disorders and also is a critical period of maturation of normal PFC function. A pPFC dysfunction that may arise due to a genetic predisposition or external influence, like stress, during the adolescent period might have an important impact on the susceptibility to mood disorders in adults.

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Highlights

- Adolescent pIPFC dysfunction increases vulnerability to helpless behavior at adulthood.
- Adult pIPFC disruption did not change vulnerability to helpless behavior.
- Helpless behavior is associated with blunted dopaminergic activity in the medial ventral tegmental area.

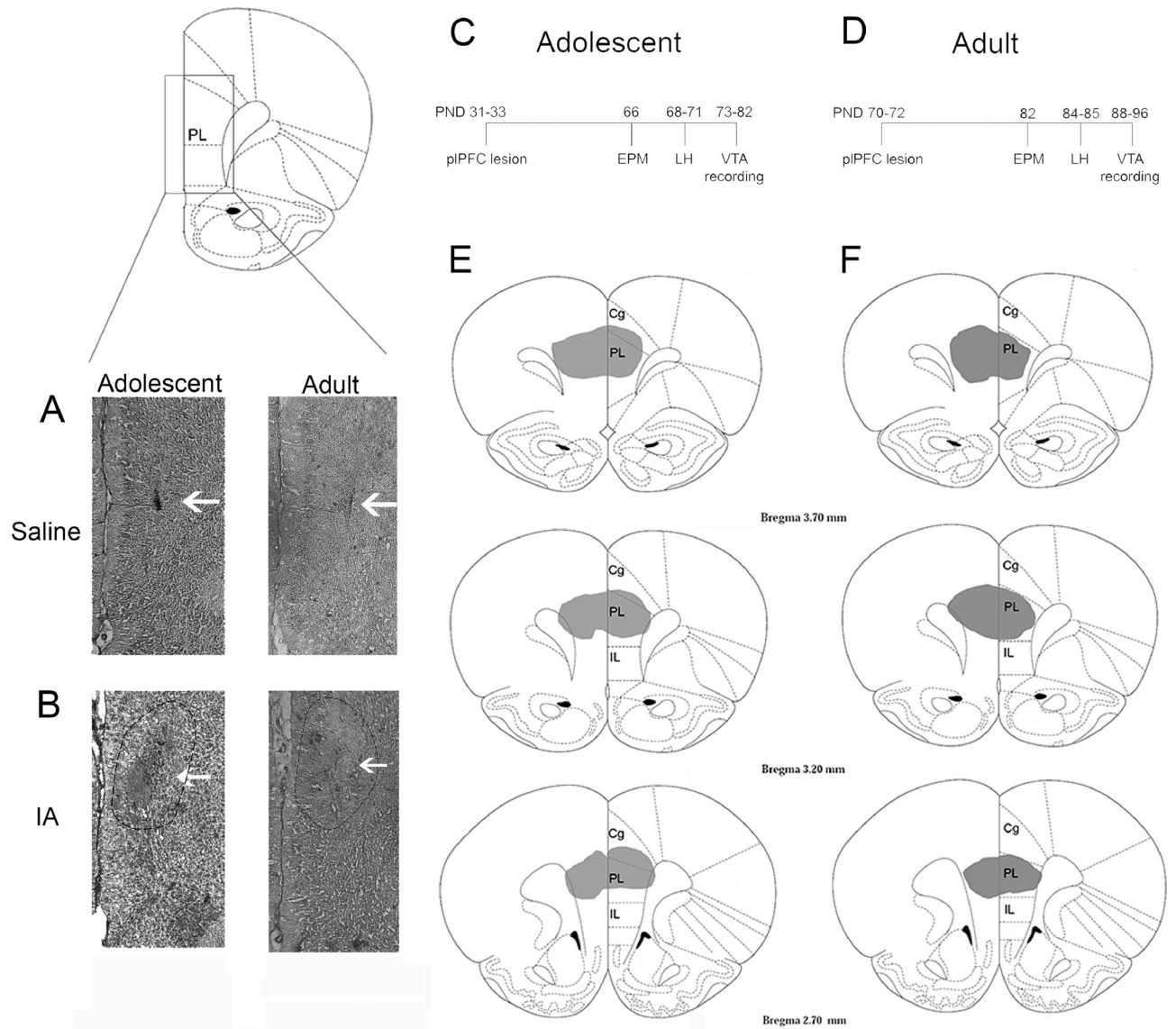


Figure 1. Schematic representation of lesion extent in the pIPFC. Representative photomicrographs of saline (A) and ibotenic acid (IA; B) injections into pIPFC of adolescent and adult rats. Time course of the adolescent (C) and adult (D) pIPFC lesion experiments. Coronal section illustration of the extent of the ibotenic acid lesion in adolescent rats (E) and adult rats (F). Cg: cingulate; PL: prelimbic PFC; IL: infralimbic PFC.

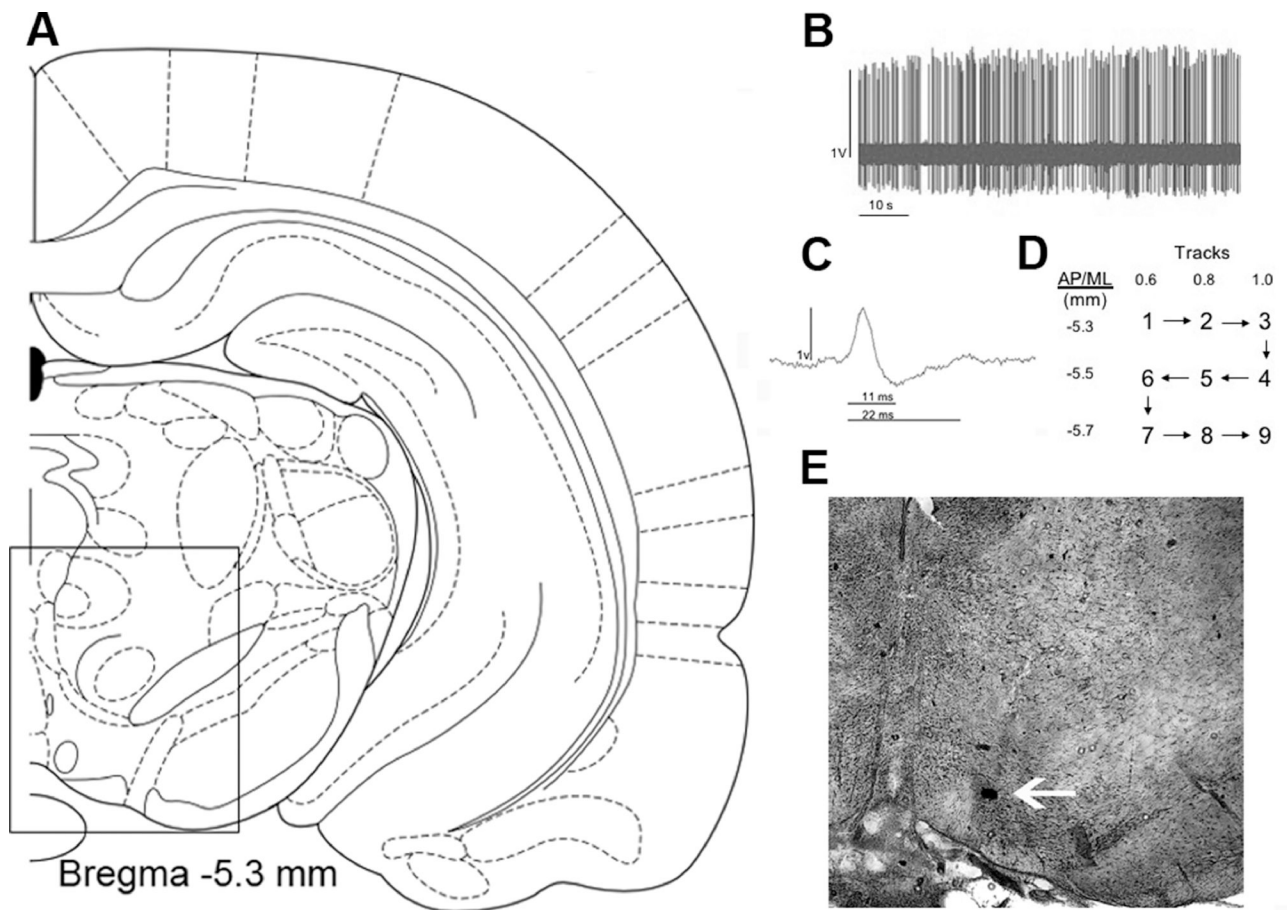


Figure 2. Electrophysiological properties of dopaminergic neuron recording in the VTA (A). Spontaneous activity tracing recorded over 1 min (B). A representative dopaminergic neuron waveform (C). The pattern of tracks performed during the recording in the VTA (D) and representative photomicrography of an electrode localization in the VTA (E). (A) was adapted from Paxinos and Watson, 2007.

Adolescent pIPFC lesion

Elevated plus maze

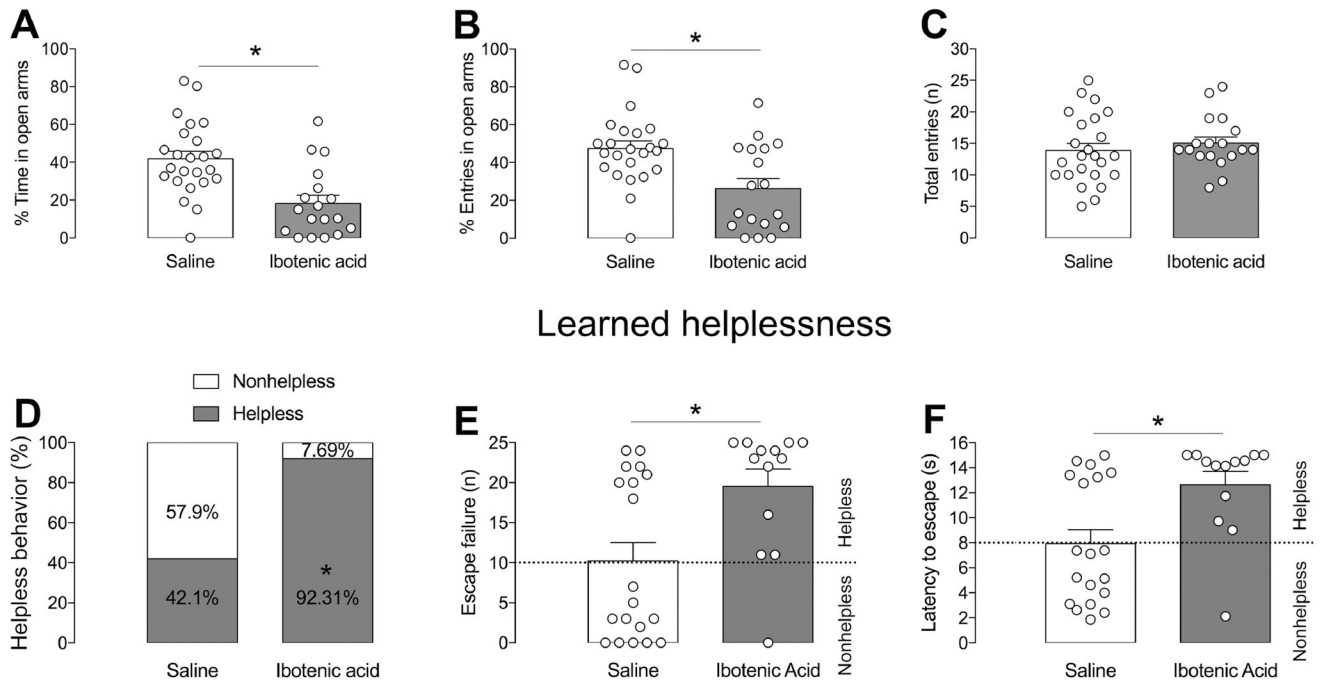


Figure 3.

pIPFC disruption during adolescence induces an anxiety-like state and increases the susceptibility to helpless behavior in adults. Adolescent pIPFC lesion (A) decreased the percentage of time (A) and the number of entries (B) into open arms of the EPM at adulthood. The total number of entries was not affected by the adolescent pIPFC lesion (C). * $p < 0.05$, t-test. In the LH experiment, adolescent pIPFC lesion increased the percentage of rats showing helpless behavior (D), the number of escape failures (E) and latency to escape (F) in the LH paradigm at adulthood (* $p < 0.05$, t-test).

Adolescent pIPFC lesion

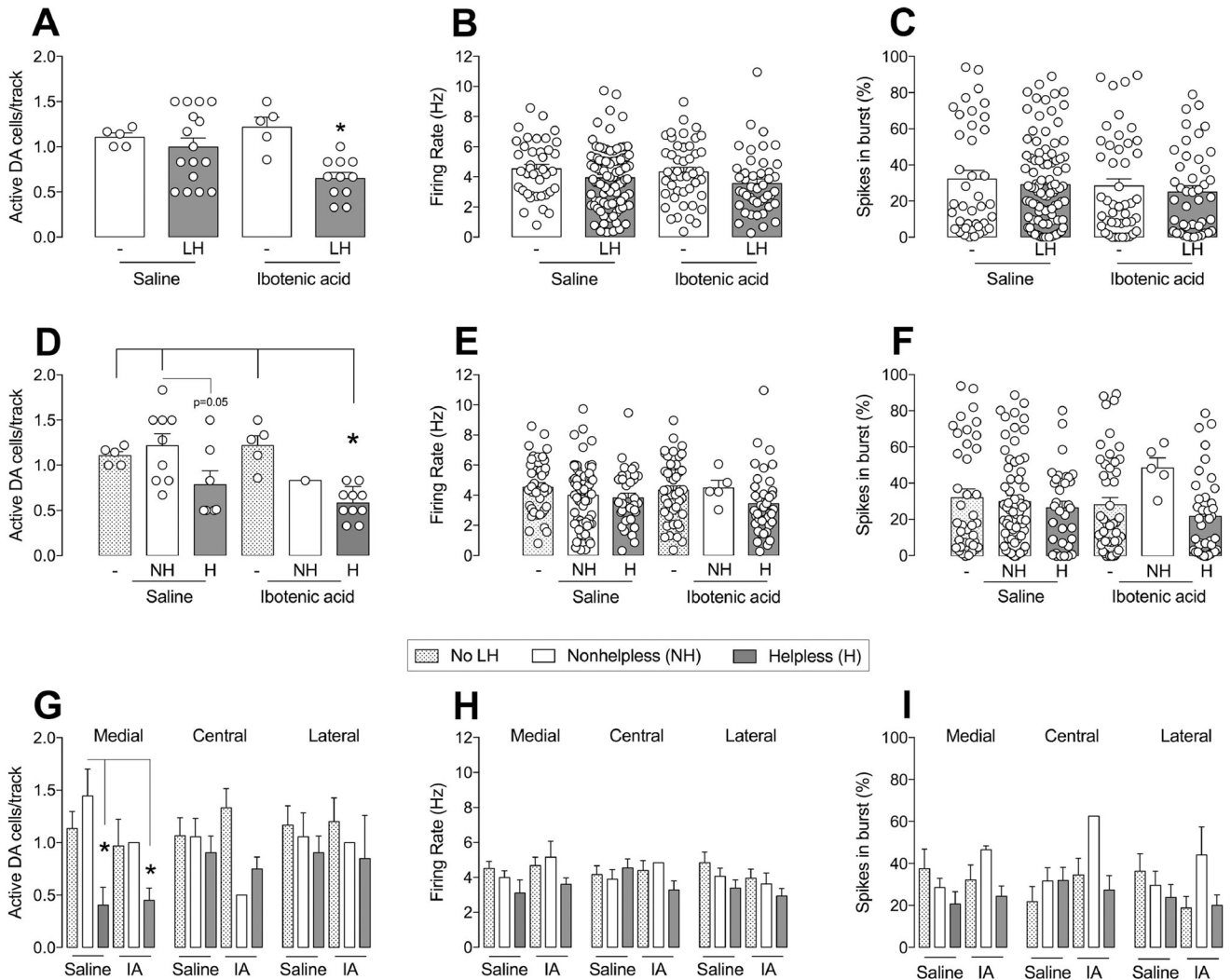


Figure 4. pIPFC disruption during adolescence decreases the number of spontaneously active DA neurons in the VTA of helpless rats. The pIPFC lesion in adolescent rats decreased the number of spontaneously active VTA DA cells/track compared to intact rats after exposure to learned helpless in adult rats (A; *p<0.05), with no change in firing rate (B) or the percentage of spikes in bursts (C). The number of VTA DA cells/track in helpless rats in the ibotenic acid group (D) is decreased in relation to no LH/nonhelpless rats of the saline groups and no LH of ibotenic acid group (*p<0.05). No statistical difference was found for firing rate (E) or in the percentage of spikes in bursts (F) of helpless/nonhelpless rats in saline and ibotenic acid groups. Helpless rats in saline and ibotenic acid group exhibit a reduction in the number of active DA cells specifically in the medial portion of VTA compared to nonhelpless and rats not subjected to LH (*p<0.05), but not in central and lateral portion (G). The medial/central/lateral VTA firing rate (H) and percentage of burst activity (I) were not affected by treatment or helpless behavior. NH: nonhelpless; H:

helpless; LH: learned helplessness; IA: ibotenic acid. * $p < 0.05$, ANOVA followed by Tukey's post hoc analysis.

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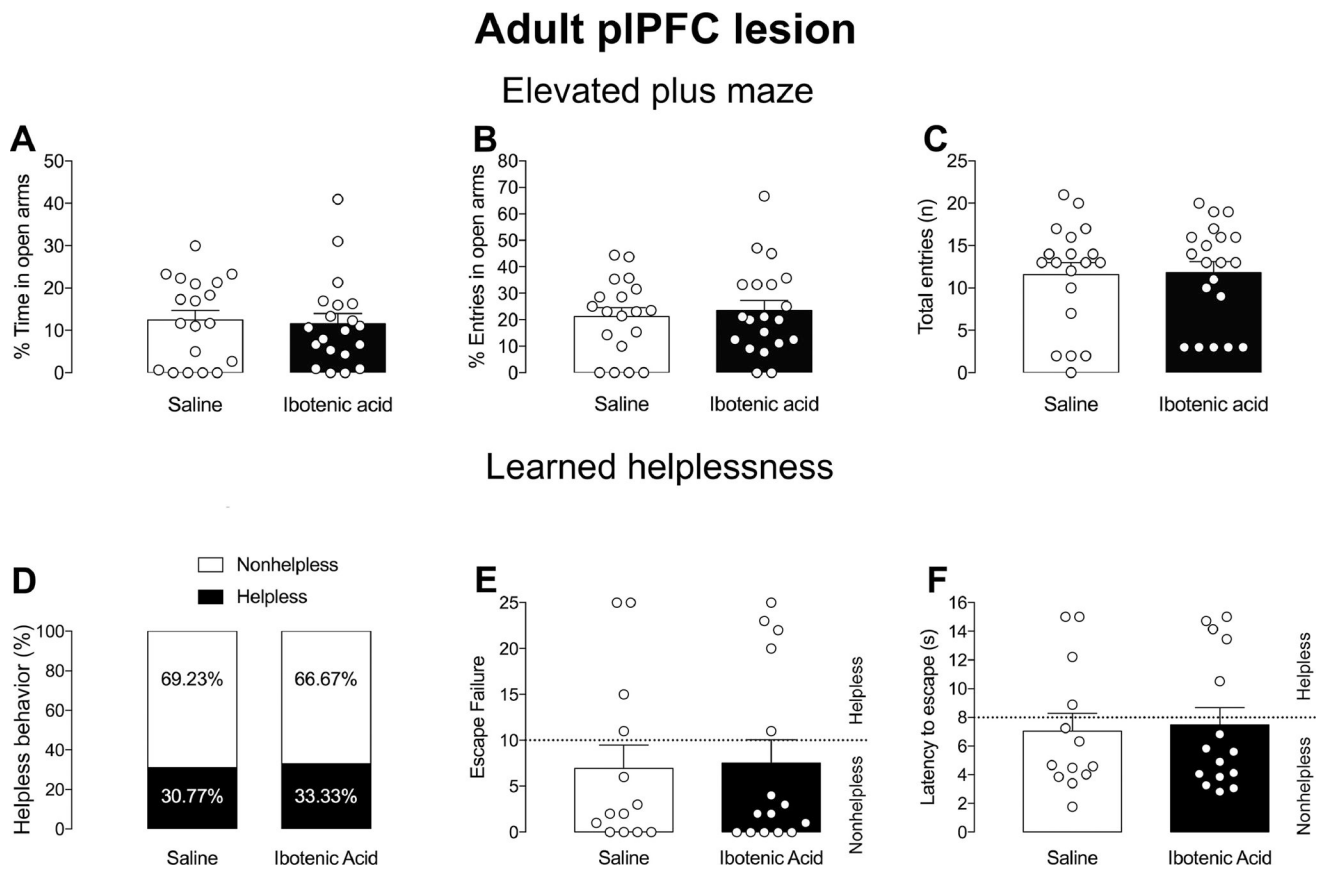


Figure 5. pIPFC lesions in adult rats do not impact behavioral response in EPM/LH. Adult pIPFC lesion did not change anxiety responses in the EPM, % Time (A), % Entrance in open arms (B) or Total entries (C). No changes in % of helpless behavior (D), number of escape failures (E) or latency to escape (F) in rats subjected to the LH paradigm.

Adult pIPFC lesion

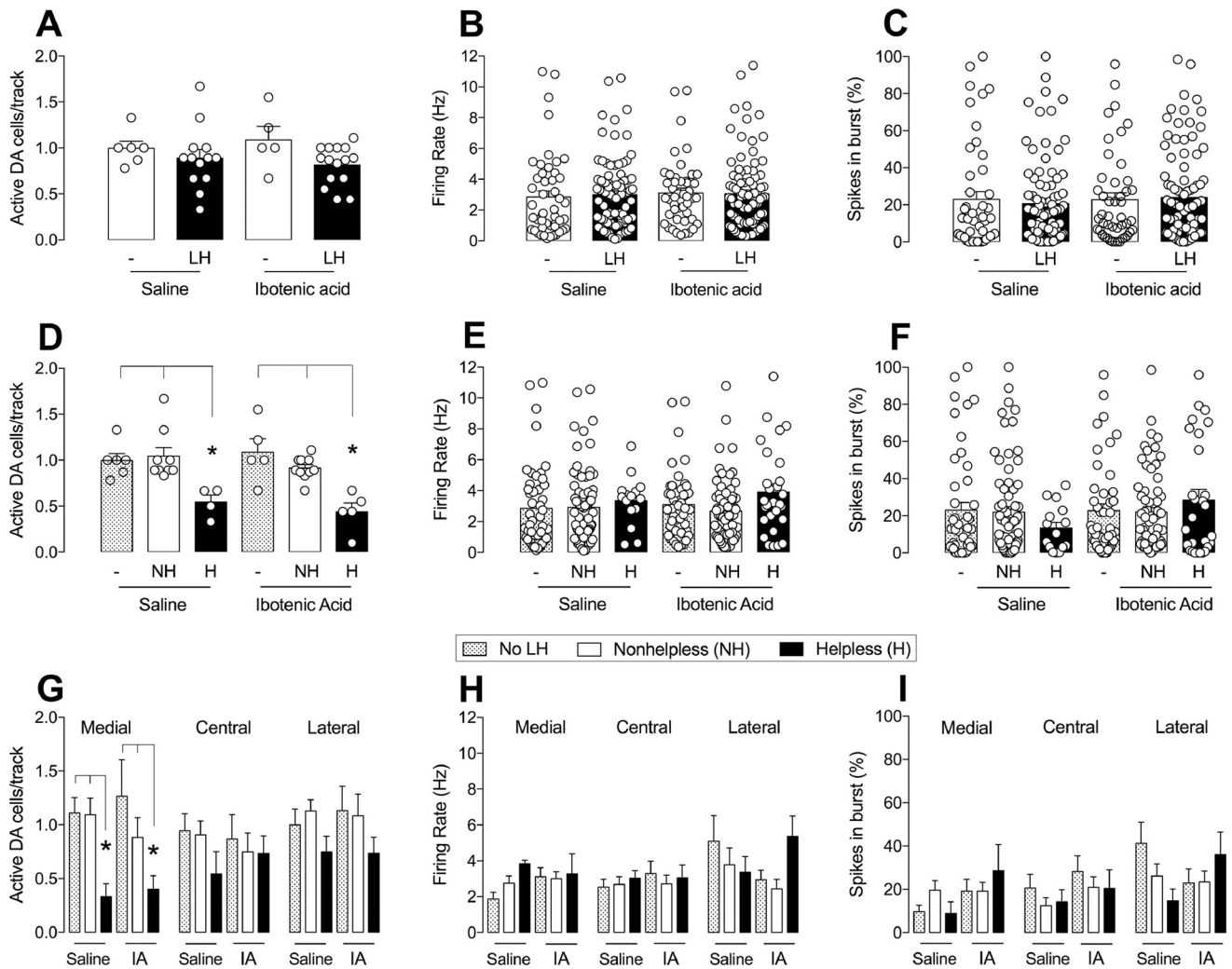


Figure 6.

The adult pIPFC lesion had no effect on VTA DA neuron activity. (A) The pIPFC lesion in adult rats did not affect the number of spontaneously active VTA DA cells/track (A), firing rate (B) or the percentage of spikes in bursts (C) after exposure to learned helplessness. The number of active DA cells/track is reduced in helpless rats in both saline and ibotenic acid groups (D) in relation to nonhelpless or animals not subjected to LH (* $p < 0.05$). No statistical difference was found for (E) firing rate or in (F) the percentage of spikes in bursts of helpless/nonhelpless rats in saline and ibotenic acid groups. Helpless rats in both treatment groups had a decrease in the number of active DA cells in the medial portion of VTA compared with nonhelpless rats and rats not subjected to LH, but not central and lateral portions (G). The medial/central/lateral VTA firing rate (H) and percentage of burst activity (I) were not affected by treatment or presence of helpless behavior. NH: nonhelpless; H: helpless; LH: learned helplessness; no LH: not subjected to learned helplessness. * $p < 0.05$, ANOVA followed by Tukey's post hoc analysis.